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Quantitative sampling of stream fish assemblages: Single- *vs* multiple-pass electrofishing

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Abstract Stream fish assemblages were sampled by multiple-pass electrofishing and supplementary seine netting in 31 sites in the Johnstone River, north Queensland and 28 sites in the Mary River, southeastern Queensland to determine the sampling effort required to adequately describe the assemblages in terms of fish abundances, species composition and assemblage structure. A significantly greater proportion of the total number of fishes present at each site was collected by the first electrofishing pass in the Mary River (46%) than in the Johnstone River (37%) and this difference was suggested to be due to higher water conductivity in the former river. The mean proportion of the total species richness detected by the first pass was also significantly higher in the Mary River than in the Johnstone River (89% and 82%, respectively). Multivariate comparisons of fish assemblage structure revealed that data collected by the first electrofishing pass poorly estimated the actual assemblage structure within a site and that up to three passes were required for estimates of assemblage structure to stabilize. This effect was evident for comparisons based on both absolute abundance and relative abundance data and was particularly marked for comparisons based on presence/absence data. This latter result suggests that, even though most species were detected on the first pass, the addition of rare species by subsequent passes had an important effect on the resultant description of assemblage structure. Supplementary seine netting had a greater effect on the determination of assemblage structure in the Mary River than in the Johnstone River. The results are discussed with reference to sampling design in studies of stream fish assemblages and a sampling protocol is recommended that enables the accurate determination of abundance, richness and assemblage structure in small- to medium-sized streams.

Key words: electrofishing, multivariate analysis, Queensland, sampling effort, seine netting.

INTRODUCTION

The quantitative collection of freshwater fishes is not an easy task and the accurate determination of fish density has been a '...weak point in much sophisticated fish research' (Zalewski 1983 p. 177). Many collecting techniques have been devised, ranging from netting using a variety of different forms (fyke, gill, seine, etc.), to poisons such as rotenone and to the use of electric currents. Each collecting method is likely to have some bias or selectivity for different taxa or sizes ranges. The poisoning of entire stream reaches appears the most effective means of acquiring accurate estimates of fish density and assemblage structure (Larimore 1961; Boccardy & Cooper 1963); however, it is rarely desirable in view of its unpredictable nature and its negative environmental effect, particularly if studies are intended to be long term.

Wiley and Tsai (1983) found that electrofishing provided better and more consistent estimates of fish densities than did seine netting. Electrofishing (electroshocking) is the use of an electrical field applied to the aquatic environment to attract and stun fish, thus enabling their capture. The earliest documented use of electric fields to capture fishes was in 1863, but its routine use in fisheries research did not occur until the 1930s (Hartley 1989). Electrofishing has been routinely used in Australia since the mid-1960s (Koehn & McKenzie 1985). One of the advantages of electrofishing is that capture does not usually result in death and fishes are able to be released back into the environment. Injury does occur occasionally, particularly to the spinal column, but often these injuries are not fatal (Spencer 1967). Our own experience suggests that electrofishing-related mortality rates for a range of species, in a range of rivers, are generally less than 5% (B. J. Pusey *et al.* unpubl. data).

The method is not without bias however. Galvanotaxic and galvanonarcotic responses vary among species and among size classes within species (Larimore 1961; Boccardy & Cooper 1963; Mahon 1980; Mahon & Balon 1980; Balayev 1981; Wiley & Tsai 1983; Koehn & McKenzie 1985). Catchability reportedly decreases with increases in the number of times an individual has

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been shocked, with this refractory period lasting between three and 24 h (Cross & Stott 1975). Electrofishing efficiency can also vary with water conductivity (Hill & Willis 1994), voltage, direction of movement of target fish within the electric field and water temperature (Regis *et al.* 1981), stream width (Kennedy & Strange 1981) and a range of other biological, environmental and technical factors (Zalewski & Cowx 1989). None the less, electrofishing is still the most effective, non-destructive sampling procedure for fishes in small- to medium-sized streams (Zalewski & Cowx 1989; Schill & Beland 1995). Unfortunately, the variability in efficiency appears to be site specific and no general rules are possible (Larimore 1961; Cross & Stott 1975; Koehn & McKenzie 1985).

The increasing focus within Australia on the sustainable management of water resources has resulted in a greater emphasis on the determination of the habitat and flow requirements of native fish species (Harris 1995). Our current research program is focused on determining the effects of stream discharge on spatial and temporal variation in fish assemblage structure and one applied outcome of this program is the provision of simple methods for use by government agencies concerned with the management of Queensland's water resources. The aim of this contribution is therefore twofold. First, we want to detail the method employed by us in current research and to allow comparison between the current and prior research (Pusey *et al.* 1993, 1995; Pusey & Kennard 1996). Second, we want to assess the effort required to accurately determine the density, species composition and assemblage structure of freshwater fishes in well-defined hydraulic units (i.e. riffle, run or pool) of streams of eastern Queensland.

METHODS

Study area

Electrofishing was undertaken in two rivers; the Johnstone River (146°0'E, 17°30'S) of northern Queensland and the Mary River (152°35'E, 25°30'S) of southeastern Queensland. Thirty-one sites were sampled by electrofishing within the Johnstone River drainage between July and September 1994. Mean water temperature during this period was 20.0 ± 0.5 (S.E.)°C and conductivity ranged from 10.5–54.7 $\mu S.cm^{-1}$ with a mean of 36.8 ± 1.8 $\mu S.cm^{-1}$ (n = 31). The streams investigated were of small to medium width, rarely deeper than 1.5 m and of varying mean water velocity and contained a wide range of types of substratum (Table 1). Areas sampled covered a wide range of habitat types (e.g. cascades, rapids, riffles, runs and pools). Sites electrofished in the Mary River (n = 28) during September and October 1994 covered a similar range of habitat types and were generally similar in structure except for slightly lower mean water velocities and a lower proportion of coarse substrata (rock and bedrock). Various types of in-stream cover (woody debris, macrophytes, etc.) were present in most sites but constituted such low proportional coverage of a site area that they were not included in Table 1. Water temperatures in the Mary River were similar to those recorded in the Johnstone River (19.8 ± 0.6°C); however, water conductivity differed greatly between the two rivers. Conductivity ranged from 161.5–1889.9 $\mu S.cm^{-1}$ (mean conductivity of 621.7 ± 78.7 $\mu S.cm^{-1}$) in the Mary River (n = 28). Importantly, water clarity was always high in both rivers (mean turbidity 1.6 ± 0.2 NTU and 3.1 ± 0.5 NTU for the Johnstone and Mary Rivers, respectively) and is unlikely to have been a significant impairment to electrofishing efficiency in this study. The mean area and length of stream electrofished was 320 ± 48.5 m^2 and 39.6 ± 4.0 m, respectively, for the Johnstone River and 272 ± 39.7 m^2 and 39.4 ± 2.8 m, respectively, for the Mary River.

Sampling procedures

Electrofishing was performed in both rivers using a portable back-pack electrofisher. In the Johnstone River, a Smith-Root Mk 12 POW electrofisher was used whereas a Smith Root Mk 7 electrofisher was used in the Mary River. Various output wave forms, voltages and pulse frequencies can be selected on the Mk 12 electrofisher but the choice is more limited in the Mk 7 model. In general, we chose to restrict the use of alternative settings in the Johnstone River (200–400 V and setting J4; frequency: 70 Hz, pulse width: 4 ms) so as to approximate the output generated by the Mk 7 model. Prior experience suggests that this output was the most effective for collecting a wide range of species

Table 1. Average structure of the habitat at each study site in the Johnstone River and Mary River

	Johnstone River	Mary River
Habitat variable	(n=31)	(n=28)
Width (m)	9.56 (7.07)	8.59 (7.92)
Depth (m)	0.37 (0.16)	0.28 (0.20)
Water velocity ($m.sec^{-1}$)	0.14 (0.10)	0.06 (0.11)
% Mud	11.3 (14.9)	5.5 (10.7)
% Sand	17.1 (20.0)	15.5 (19.1)
% Fine gravel	16.1 (12.7)	21.3 (9.6)
% Gravel	12.2 (13.0)	27.4 (14.7)
% Cobbles	11.0 (10.2)	23.9 (17.4)
% Rock	16.5 (17.8)	6.3 (12.3)
% Bedrock	16.3 (24.3)	0.3 (0.9)

The values given are the means (± 1 SD). Substrate values are given as the mean proportion (%) of the substrate for each site.

within a range of different rivers and habitat types. Both electrofishers used a standard Smith-Root anode (25 cm diameter ring attached to a 2 m pole) and cathode (3.2 m wire cable).

Electrofishing was conducted within discrete hydraulic habitat units (i.e. riffle, run or pool) within a stream reach. Prior to electrofishing, block seines (9 mm stretched mesh size) were placed at the top and bottom of the study site. The bottom of the net was weighed down with substratum to prevent the movement of fishes either into or out of the study site. The bottom seine contained a central bag or purse which was always positioned in the thalweg. Nets were deployed simultaneously whenever possible, otherwise the bottom net was positioned first. Approximately 20 min were allowed to elapse prior to commencing electrofishing in order for fish to resume normal behaviour.

The operator and one assistant then commenced electrofishing, using short, intermittent pulses, ensuring that all of the enclosed area was electrofished once. This procedure was considered to represent a single electrofishing pass. In the case where stream width was less than 4 m, electrofishing commenced at the upstream seine and proceeded to the bottom seine. In wider streams, the operator moved through the study site in a zig-zag fashion. All stunned fish were netted and placed immediately in stream water in a 70 L plastic container towed behind the assistant or, in the case of large fish (e.g. therapontids, plotosids and anguillids), immediately transferred to an additional 70 L container on the stream bank. Fish present in the bag of the bottom net were removed at the completion of each pass and were counted as part of each respective electrofishing pass.

Approximately 15 min were allowed to lapse before the second pass commenced. All subsequent passes proceeded in the same manner until few or no more fish were collected by electrofishing. A maximum of five passes was required for complete depletion in both rivers except for two sites in the Mary River. The rate of depletion of some small agile species (*Retropinna semoni, Melanotaenia* spp. and *Craterocephalus* spp.) from the study site with each sequential pass was often observed to be lower than the remaining assemblage members. Whenever possible, seine netting (mesh size 9 mm) was used to collect all of these remaining schooling fishes. Snorkelling was occasionally undertaken to assess the success of the sampling, and in most cases, few fish were observed. If many fish were observed to be present, then electrofishing and seining continued. Most fish collected were returned to the study site alive after identification and measurement. The duration of the sampling procedure described above was commonly between two and four hours in the Johnstone River and one and three hours in the Mary River with duration being determined mainly by the size of the study site, the number of fish present and the number of electrofishing passes conducted.

Data analysis

Univariate analysis of abundance and species richness

The total number of fishes collected from each study site after all electrofishing passes had been completed, and after any additional seine netting, was taken to represent the total number of fishes present within each site (N_T). The cumulative total abundance collected up to pass i was represented by N_i. The proportional contribution of each cumulative pass was estimated (N_i/N_T). Therefore, at each site, the cumulative abundance was standardized by the total site abundance. The proportional contribution of seine netting to N_T was similarly standardized. Six electrofishing passes, as opposed to five, were necessary to achieve full depletion in only two of the 28 sites in the Mary River. In order to simplify analyses, the sixth pass was added to the fifth pass in these two sites. This was not considered problematic because the fish collected on the sixth pass constituted less than 5% of the total collected at each site and consisted of only one species. Initial analysis of the relationship between variances and means indicated heterogeneous variances. To stabilize the variances, the standardized cumulative abundances were log (x + 1) transformed prior to further analyses (Underwood 1997).

Preliminary analysis indicated that the partial correlations obtained from the sums of squares and crossproducts error matrix between cumulative passes, were significant ($P < 0.0001$, d.f = 57). A test of sphericity on this matrix was also significant ($P < 0.0001$, d.f. = 14). These analyses indicated interdependency of the within-river effects (standardized cumulative passes), therefore a repeated-measures Analysis of Variance (SAS Institute Inc. 1989) was performed. For all significant standardized cumulative pass–by–river interactions, orthogonal contrasts between the nth level cumulative pass and the total collected by electrofishing were performed for each river. A single contrast between the first pass of each river relative to the total cumulative abundance for each river was also performed.

Total species richness was taken to equal the number of species collected at an individual site once all fishes had been removed by both electrofishing and seine netting. These data were treated similarly to abundance data in that we estimated the cumulative proportion of the total richness detected by each electrofishing pass and all preceding passes. A similar repeated-measures analysis was used to analyse variation using log (x + 1) transformed cumulative species richness. The effect of habitat structure on the efficiency of the first pass was assessed by correlation

analysis of N_I/N_T (arcsine transformed) against the mean of a range of variables describing habitat structure including stream width, depth, water velocity, the proportional contribution of substrate types listed in Table 1 and the proportional areal cover of cover elements including macrophyte beds, small and large woody debris, leaf litter and root masses.

Multivariate analysis of assemblage structure

In this analysis we were interested in assessing whether our determinations of the fish assemblage structure at each site changed significantly with increasing sampling effort. To this end we examined data from the first electrofishing pass, first plus second electrofishing pass and so on and compared the assemblage structures determined from each such combination against the assemblage structure determined from all electrofishing passes and any supplementary seine netting (= total assemblage). Differences in fish assemblage structure were examined by two different methods for each of three separate data sets. These data sets were generated for each river and consisted of: (i) species abundance data (no standardization), (ii) relative abundances (standardized by site total), and (iii) species presence/absence data. The first two generated data sets were log (x + 1) transformed to downweight the abundance of a few numerically dominant species. All analyses were based on the Bray–Curtis dissimilarity measure (Bray & Curtis 1957). Simulation studies (Faith *et al.* 1987) have indicated that this dissimilarity measure is an effective measure of ecological distance.

The first analysis used a Mantel test (Mantel 1967; Manly 1991) to compare the site by species dissimilarity matrix for each cumulative pass with the matrix derived for the total assemblage, for each of the three generated data sets within each river. The Mantel test (a randomization procedure) uses a standardized dissimilarity matrix obtained by subtracting the matrix minimum and dividing by the matrix range; it produces an average distance measure between each matrix pair. The test matrix was compared with one thousand randomized test matrices using the same standardized distance measure to obtain each Mantel test statistic.

All possible between-pass Mantel distances (i.e. pass 1 *vs* pass 1 + 2,.....pass 1 *vs* all passes plus seine netting, pass 1 + 2 *vs* pass 1 + 2 + 3,.... pass 1 + 2 *vs* all passes plus seine netting, etc.) were calculated for all possible between-pass comparisons. These Mantel distances were then used to form new matrices for each of the three data sets for each river. These new matrices were then used in subsequent Semi-Strong Hybrid Multidimensional Scaling (SSHMDS) ordinations (Belbin 1991), as implemented in the SSH procedure in PATN (Belbin 1995), to generate plots representing the differences between all passes. These ordinations were based on two dimensions with stress levels consistently less than 0.05.

The second analysis was based on comparisons of ordinations for each data set within each river. In this case, the underlying matrix of Bray–Curtis dissimilarities was derived from the original site by species matrix and ordination (SSHMDS) and was performed using the SSH procedure in PATN (Belbin 1995). Fifty random starts were performed and the randomization which showed the lowest stress level was chosen for further examination. Each ordination was performed for three dimensions because any further increase in the number of dimensions did not greatly decrease the stress levels, all of which were below 0.15. Resultant ordinations were compared using a Procrustes analysis (Gower 1971; Sibson 1978), in which the ordination plot obtained for each successive cumulative pass (the test plot) is rotated, scaled or reflected to fit the ordination plot generated for the total assemblage (the target plot) in order to minimize the sum of squared distances between the samples. This yields a measure of overall fit, the Root Mean Squared Residual (RMS), which is the square root of the mean of the squared distances between corresponding samples in the fitted and target ordinations (Sibson 1978). This method quantified the match between the multidimensional scaling ordinations of each cumulative pass and the total assemblage. The RMS obtained from this procedure is a composite of the differences in the underlying dissimilarity matrices and the ability of the ordination process to fit these distances in a dimensionally reduced space.

We suggest that both the Mantel analysis and the Procrustes analysis are informative. Mantel's analysis directly tests the 'distance' between two dissimilarity matrices. Ordination, which most researchers use as an appropriate means to examine variation in species abundances, is based on interpretation of the underlying dissimilarity matrix, except that the interpretation occurs in a dimensionally reduced space with a potential consequent loss of information. Therefore, it is useful to directly compare the differences in the matrices (i.e. Mantel comparisons of the standardized distances) and to indirectly compare differences when the underlying structure is displayed in a reduced set of dimensions (i.e. RMS comparisons from the Procrustes analysis).

RESULTS

Abundance

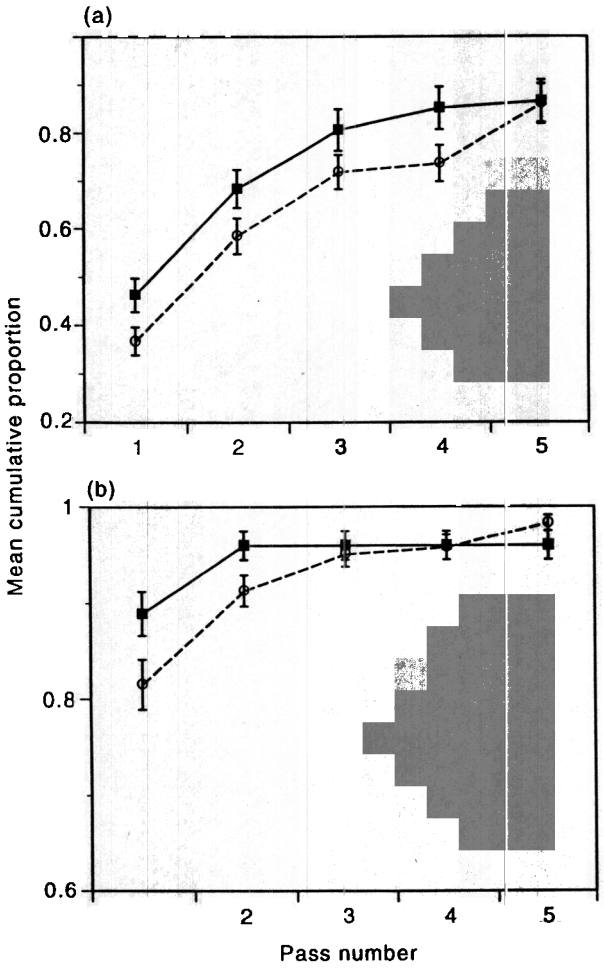
The number of fish collected from each site varied between 90 and 200 and 128–190 for the Johnstone and Mary Rivers, respectively. The exact F statistic (Wilks Lambda) from the repeated measures ANOVA, indicated a significant cumulative pass-by-river inter-

Table 2. *F* values and their associated levels of significance for a repeated-measures Analysis of Variance of within river (cumulative passes) and between river variation in log(x+1) transformed proportional cumulative abundance and species richness

		Cumulative abundance		Cumulative species richness	
Source of variance	d.f.	*F*	*P*	*F*	*P*
River	1	2.14	NS	1.17	NS
Site (River)	57				
Pass	4	136.20	<0.0001	13.12	<0.0001
Pass × River (Error 1)	5	10.75	<0.0001	4.79	<0.001
Contrast 1:					
Johnstone R.(P1) *vs* Mary R.(P1)		5.38	<0.05	9.91	<0.005
Contrast group 2 (Johnstone R.):					
P1 *vs* P5		307.02	<0.0001	52.09	<0.0001
P2 *vs* P5		92.28	<0.0001	21.64	<0.0001
P3 *vs* P5		88.47	<0.0001	10.57	<0.005
P4 *vs* P5		78.19	<0.0001	7.47	<0.05
Contrast group 3 (Mary R.):					
P1 *vs* P5		121.79	<0.0001	11.90	<0.005
P2 *vs* P5		51.81	<0.0001	0	NS
P3 *vs* P5		13.99	<0.001	0	NS
P4 *vs* P5		3.96	NS	0	NS
Pass × Site (River) (Error 2)	53				

See text for further explanation of contrasts. Cumulative electrofishing passes as denoted by P1 to P5 where the numeric value is the number of passes (i.e. P5 = pass 1 + 2 + 3 + 4 + 5).

NS, not significant.



action ($P < 0.0001$, Table 2). Contrasts between the *n*th cumulative pass and the total indicated that for the Johnstone River, each electrofishing pass significantly increased the mean cumulative proportion of the total abundance ($P < 0.0001$ for all comparisons, Table 2, Fig. 1a). In the Mary River the first three passes contributed significantly to the total proportion of fish collected ($P < 0.001$) but the remaining passes did not significantly alter the total number of fish collected by electrofishing ($P > 0.05$, Table 2, Fig. 1a). Electrofishing efficiency (proportion of fish collected on the first pass) differed significantly between the two rivers ($P < 0.05$, Table 2) with a higher proportion of fish collected on the first pass in the Mary River than in the Johnstone River (mean proportion = 0.46 ± 0.04 SE and 0.37 ± 0.03 SE, respectively) (Fig. 1a). Seine netting, after completion of all electrofishing passes, collected a mean of 14% of the total number of fishes present at each site at those sites in which it was used. Electrofishing efficiency was not significantly correlated ($P > 0.05$) with any parameter describing habitat structure in either river (Table 1).

Fig. 1. Sequential increase in the mean cumulative proportion (SE) of (a) total abundance and (b) total species richness collected by each electrofishing pass in the Mary and Johnstone Rivers. (■) Mary River; (○) Johnstone River. Data from supplementary seine netting is not included in this figure and hence mean cumulative proportions do not sum to 1.

Species richness

The number of species of fish collected from each site varied from 3 to 14 and 2 to 14 for the Johnstone and Mary Rivers, respectively. The first electrofishing pass detected a significantly higher mean proportion of the total number of species in the Mary River than in the Johnstone River (0.89 ± 0.02 SE and 0.82 ± 0.03 S.E., respectively) ($P < 0.005$, Table 2, Fig. 1b). The exact F statistic from the repeated measures ANOVA, indicated a significant cumulative pass–by–river interaction ($P < 0.001$). Contrasts between the nth cumulative pass and the total indicated that for the Johnstone River, each electrofishing pass significantly increased the mean cumulative proportion of the total species richness collected ($P < 0.005$ for all comparisons, Table 2, Fig. 1b). In the Mary River however, no additional species were collected after the first two passes (Table 2, Fig. 1b).

Seine netting collected fewer species than did electrofishing because the majority of species had been detected already and removed by electrofishing. Moreover, on only three of the 22 occasions in which seine netting was employed in the Johnstone River did it collect a species not already collected by electrofishing. Six of the 14 occasions in which seine netting was employed in the Mary River resulted in the detection of any additional species, but in all cases except one, this was limited to one additional species.

Multivariate analysis of assemblage structure

The Mantel randomization tests showed, for all data sets (absolute abundance, relative abundance and presence/absence) from both rivers, that the standardized difference between the matrix of site dissimilarities for each cumulative pass and the total assemblage dissimilarity matrix was significantly different from random ($P < 0.001$). This indicated that, for both rivers, a single pass was sufficient to indicate that the spatial pattern of fish assemblages deviated significantly from random. Further sampling effort did not alter this conclusion. However, it is evident in Fig. 2a, that further sampling effort did reduce the magnitude of the Mantel statistic and therefore the addition of new information from each sequential pass resulted in a better representation of the total assemblage structure.

The Procrustes analyses for both the Mary River and Johnstone River data sets revealed a similar pattern of decreasing RMS values with the addition of data from the second pass, followed by little change with the addition of data from the third pass, especially in the Mary River (Fig. 2b). The presence/absence data set was more influenced by the addition of rare species and therefore generally maintained higher Mantel distance values and RMS values than those comparisons based on abundance or relative abundance (Fig. 2). These data indicate that after three passes, little change in the determination of fish assemblage structure was evident. The abundance and relative abundance data sets for the Mary River maintained higher Mantel distance values and RMS values after completion of all electrofishing passes than those of the Johnstone River (Fig. 2), suggesting that supplementary seine netting influenced the determination of total assemblage structure more in the Mary River than in the Johnstone River.

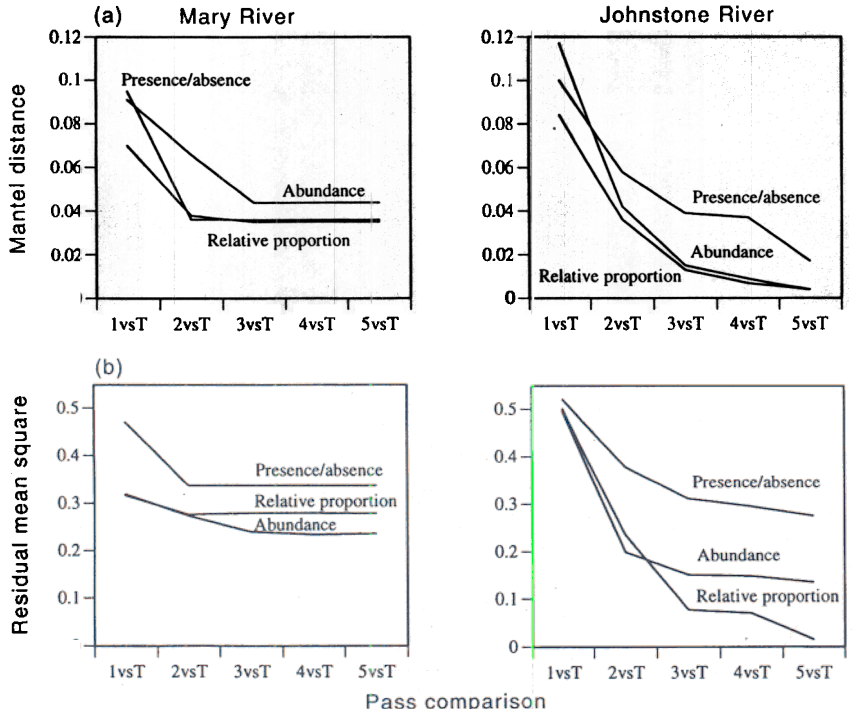


Fig. 2. Changes in (a) Mantel scores (cross matrix correlations) and (b) residual mean square values with increasing number of electrofishing passes in the Mary and Johnstone Rivers for comparisons based on abundance, relative abundance and presence/absence data.

Ordination plots of all pairwise Mantel comparisons between sequential passes, summarize the changes in assemblage structure for both rivers (Fig. 3) and support the conclusions drawn from the previous two analyses. In the Johnstone River, each successive pass had a decreasing effect on the determination of assemblage structure. The addition of data from the second and third passes most strongly affected the determination of total assemblage structure, and seine netting added very little new information. Data from the Mary River catchment shows similar trends with the greatest amount of new information being added with the second pass and subsequent passes adding little. Although the Mantel tests indicated that the difference between the final electrofishing pass and the total assemblage are closely 'correlated' in both rivers, Fig. 3 suggests that supplementary seine netting added important new information in the Mary River.

DISCUSSION

Meaningful environmental monitoring programs must be based on both accurate and precise sampling methods (Maher *et al.* 1994). Unfortunately, this increases the costs of such programs due to the increased personnel and time required to quantify abundances, assemblage composition and the relationships of both to the habitat and environment (Sheldon 1984). In the past, attention has been focused on determining whether standard collection and analytical methods may be adapted to allow a more

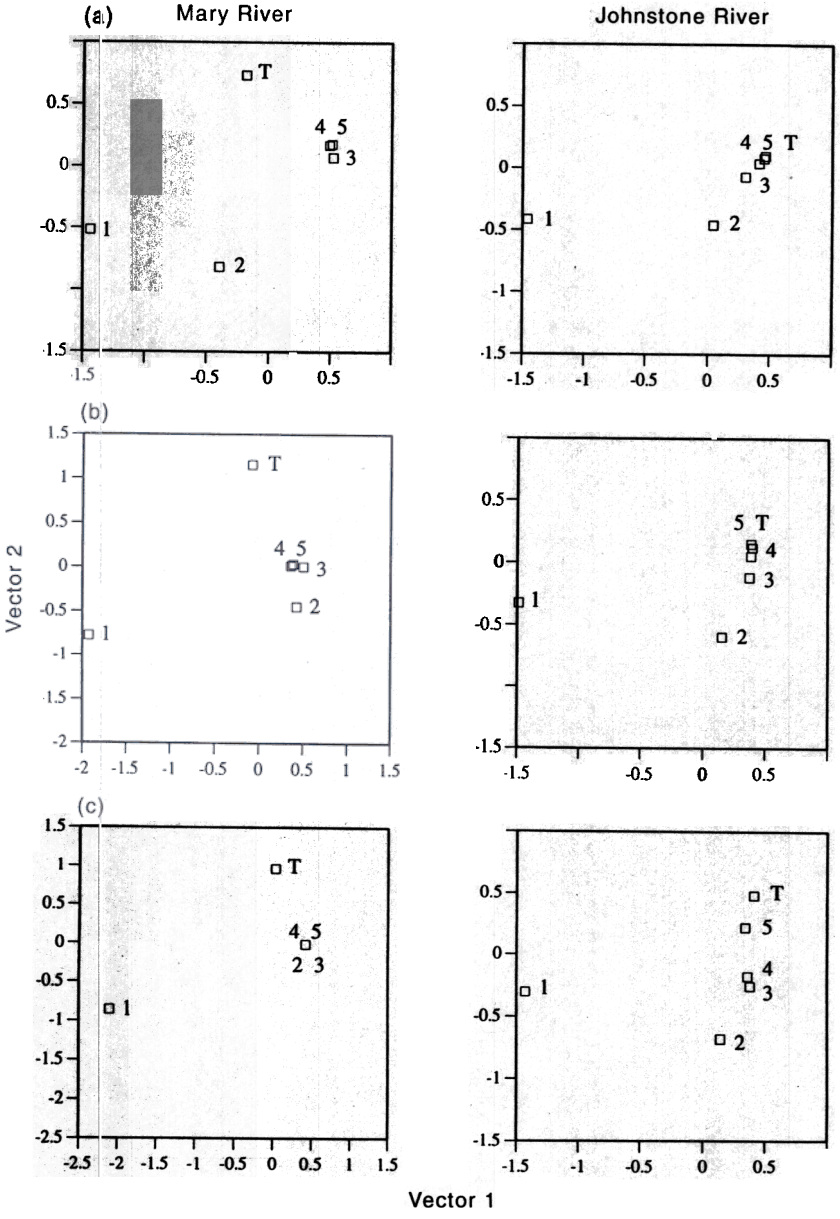


Fig. 3. Ordination plots of changes in the estimation of fish assemblage structure with increasing number of electrofishing passes. (a) Abundance, (b) relative proportion, and (c) presence/absence. The matrix upon which each ordination is based consisted of Mantel distance scores for each possible comparison (i.e. pass 1 *vs* pass 1 + 2 ... pass 1 *vs* all passes plus seine netting, pass 1 + 2 *vs* pass 1 + 2 + 3 ... pass 1 + 2 *vs* all passes plus seine netting, etc.) Each data point represents the number of passes (i.e. 2 = first plus second pass, 3 = first plus second plus third pass) and T denotes the total assemblage (all electrofishing passes plus supplentary seine netting). Stress levels for all ordinations were below 0.05.

rapid but still meaningful description of biological communities (Storey *et al.* 1991; Armitage & Pardo 1995; Marchant *et al.* 1995; Ruse & Wilson 1995; Resh *et al.* 1995). With the exception of Harris (1995) and Growns *et al.* (1996), Australian studies that address such issues in freshwaters have focused on macroinvertebrates. Elsewhere, where electrofishing has had a longer history and freshwater fisheries are traditionally a more prominent issue, this is not the case.

For example, comparisons have been made of the relative efficiencies of electrofishing and other census techniques in North America (see Introduction). Vadas and Orth (1993) compared electrofishing and seine netting in an open sampling design (only the bottom of the study area blocked by a seine) and found that both sampling techniques provided concordant fish assemblage patterns for riffles and runs, but pool samples differed substantially in species diversity and dominance patterns. They further suggested that both methods should be used together wherever possible. In some circumstances (i.e. large expanses of moderately deep water over a fine substrate with little in-stream cover), seine netting is effective (B. J. Pusey *et al.* unpubl. data). However, in more heterogeneous habitats, electrofishing is able to detect more species than does seine netting (Pusey & Kennard 1996). The relative efficiencies of each method appear to be related to the preferred habitat of individual species (e.g. benthic *vs* pelagic) (Vadas & Orth 1993). In a study of habitat use by young brown trout and Atlantic salmon, surface observations, underwater diving and electrofishing gave widely disparate information (Heggenes *et al.* 1990). Electrofishing was more effective than visual methods in shallow areas with greater water velocities and coarse substrates.

How effective is single-pass electrofishing at describing fish assemblage structure? The results of univariate comparisons of cumulative abundance and species richness, and multivariate comparisons of assemblage structure for abundance, relative abundance and presence/absence, all suggest that a single pass does not adequately describe the total assemblage because significant new information was added by further electrofishing passes. In the Johnstone River, up to three passes were required for estimates of assemblage structure to stabilize (as assessed by significant differences for contrasts in the repeated measures ANOVA and substantial changes in Mantel scores and RMS values). This was particularly evident for comparisons based on presence/absence data. The Johnstone River, like most of rivers of the Wet Tropics region, contains a high number of species, many of which occur at low densities (Pusey & Kennard 1996). Thus, the ability to detect rare species appears to be important in the final determination of assemblage structure. Fewer passes were required for a stabilized description of assemblage structure in the Mary River, probably because of increased capture rates and species detection in the first passes. Despite this apparent improvement over that observed in the Johnstone River, relatively higher Mantel scores and RMS values on the last pass suggest that supplementary seining after electrofishing provided more new information than it did in the Johnstone River.

Angermeier and Smogor (1995) reported that in order for estimates of species richness and relative abundances to stabilize, then single-pass electrofishing must be conducted over stream lengths of between 22 and 67 times the stream width. This high sampling effort was in part related to different habitat usage by different species (and therefore as many habitat elements as possible needed to be sampled), but was most strongly related to overall low population densities of fishes. Rare species required greater effort to detect. Estimates of relative abundance required less effort than determination of species richness (Angermeier & Smogor 1995). Previous research on fish assemblages in eastern Queensland (Pusey *et al.* 1993, 1995; Pusey & Kennard 1996) used a single-pass electrofishing approach which differed from the approach described here in that the single pass occurred over 200–300 m of stream. In most cases, the length of stream sampled approximated about 20–30 stream widths. In this regard, estimates of species richness and relative abundances were probably adequate given that described patterns of spatial and temporal variability were often strongly expressed. However, when a study reach is long and contains more than one discrete hydraulic unit, it may be difficult to determine the degree to which habitat structure influences the presence of a species and its abundance. For example, a stream reach with a length of 60 times its width will have about five riffles and five pools (Keller & Melhorn 1978) and estimates of habitat structure over that reach derived from the means of a number of replicated measures would not accurately describe the study reach.

Water conductivity has been shown to be a very important determinant of electrofishing efficiency (Zalewski & Cowx 1989; Hill & Willis 1994). Conductivities measured in the Mary River were almost 20 times higher than in the Johnstone River. Given the absence of major differences in the size or structure of the study sites and proficiency of the two operators (BJP and MJK), between-river differences in electrical conductivity are the most probable cause of the observed differences in electrofishing efficiency. These data suggest that if studies of stream fishes were to rely on single-pass electrofishing only, then considerable effort to quantify the bias or error in electrofishing efficiency associated with spatial and temporal variation in water conductivity would be needed. Variation in electrofishing efficiency associated with variation in habitat structure has been reported previously (Wiley & Tsai 1983; Heggenes *et al.* 1990; Vadas & Orth 1993) and again suggests that substantial quantification of the

extent and nature of this bias would be needed if single-pass electrofishing was the sole means of sampling stream fishes. We were initially surprised not to detect any relationship between habitat structure and electrofishing efficiency; however, it is of little concern to the outcomes of this study as we sampled a very similar array of habitat types in both rivers (B. J. Pusey *et al.* unpubl. data). Specific differences in susceptibility to capture by electrofishing as indicated by the results of this study would also necessitate quantification of this effect. The estimation of species richness per site by a single pass initially appears adequate (collecting 82 and 89% of species in the Johnstone River and Mary River, respectively) and if we were only interested in spatial or temporal variation in the number of species present, this may be acceptable. To assume a constant or randomly fluctuating catchability may however, be a major problem (Mahon 1980). Semiquantitative sampling in species-rich assemblages and in heterogeneous habitats has been found to be ineffective (Mahon 1980; Zalewskii 1983) and Mahon (1980 p. 357) suggested that the most appropriate way of decreasing error was to increase the total effort and that this was best done by '... increasing the number of fishings used in the estimate'.

The desired outcome of a sampling design is the most important point that needs to be addressed at the beginning of any research or monitoring program. For example, data collected by single-pass electrofishing will only allow the detection of significant and real spatial and temporal changes in abundance of most species within an assemblage if the probability of capture on the first pass is constant for all passes or variation in abundances is pronounced. This would apply also to examination of species richness and assemblage structure. For example Jowett & Richardson (1996) were able to use data collected by single-pass electrofishing in an analysis of the distribution and abundance of freshwater fish in New Zealand rivers because all species were collected with equal probability on the first pass. The results of the present study suggests that this is not the case in the two rivers studied here given that significant changes in species richness and substantial changes in the estimation of assemblage structure occurred with the addition of data from successive passes. If however, the desired outcome was a determination of species richness and relative abundances, then single-pass electrofishing over a long length of stream may be adequate (Angermeier & Smogor 1995). The main aim of a survey of the freshwater fish fauna of rivers of the wet tropics region of Queensland (Pusey & Kennard 1996) was to estimate species richness and detect geographical differences in assemblage composition. Single-pass electrofishing over about 200 m of stream at each site was employed to achieve these aims. This allowed the sampling of many microhabitats (i.e. log jams, macrophyte and leaf litter beds, etc.) and an increased number of mesohabitat units (i.e. pool, run and riffle) within a stream reach. Additional sampling methods (seining, gill netting, angling and underwater snorkelling) rarely detected additional species in this study (Pusey & Kennard 1996). Some assessment of the importance of rare species is needed under a single-pass protocol, particularly if rare species are an important component of a region's fish fauna. For example, 25 of the 66 species recorded by Pusey and Kennard (1996) collectively comprised less than 1% of the total number of fishes collected. Rare species may be downweighted or omitted from subsequent analyses depending on the focus of the research but this may be problematic if such species are of high conservation or ecological significance.

In conclusion, this study has shown that estimates of fish species richness and abundance, and hence assemblage structure, varied according to the amount of effort expended in their determination. Furthermore, the amount of effort required to achieve an accurate determination of these parameters also varied among study rivers. Accordingly, we recommend that the minimum protocol for sampling stream fishes should include the use of top and bottom block seines and at least three electrofishing passes followed by seine netting where practicable. If analyses are limited to an examination of the distribution of fishes within watersheds only (i.e. presence/absence), the area sampled is relatively small (i.e. individual riffles or pools) and the detection of rare species is considered important, then four electrofishing passes may be necessary. Single-pass electrofishing over an extended length of stream may improve the ability to detect rare species but may compromise the ability of the investigator to clearly examine those factors responsible for determining spatial patterns of distribution.

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